UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/624,631	07/21/2003	Luigi Grasso	MOR-0241/HD0002 US	9935

23377 7590 08/29/2007

WOODCOCK WASHBURN LLP
CIRA CENTRE, 12TH FLOOR
2929 ARCH STREET
PHILADELPHIA, PA 19104-2891

EXAMINER	
HILL, KEVIN KAI	

ART UNIT	PAPER NUMBER
1633	

MAIL DATE	DELIVERY MODE
08/29/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/624,631	GRASSO ET AL.	
	Examiner	Art Unit	
	Kevin K. Hill, Ph.D.	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address. --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *August 6, 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-25,27,29-43,47-66 and 68-72* is/are pending in the application.

4a) Of the above claim(s) *1-18,21-25,27,29-43,47-66 and 68-71* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *19,20,28 and 72* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Detailed Action

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on August 6, 2007 has been entered.

Amendments

In the reply filed August 6, 2007, Applicant has amended Claim 19. Claims 1-18, 21-25, 27, 29-43, 47-66, 68-71 are pending but withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a non-elected invention, there being no allowable generic or linking claim. Claims 26, 44-46 and 67 were previously cancelled. Claims 19-20, 28 and 72 are under consideration.

Information Disclosure Statement

Applicant has filed an Information Disclosure Statement on April 18, 2007 that has been considered. The signed and initialed PTO Form 1449 is mailed with this action.

Examiner's Note

Unless otherwise indicated, previous objections/rejections that have been rendered moot in view of the amendment will not be reiterated. The arguments in the August 6, 2007 response will be addressed to the extent that they apply to current rejection(s).

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

1. **Claims 19-20, 28 and 72 stand rejected under 35 U.S.C. 112, first paragraph**, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

While determining whether a specification is enabling, one considers whether the claimed invention provides sufficient guidance to make and use the claimed invention. If not, whether an artisan would have required undue experimentation to make and use the claimed invention and whether working examples have been provided. When determining whether a specification meets the enablement requirements, some of the factors that need to be analyzed are: the breadth of the claims, the nature of the invention, the state of the prior art, the level of one of ordinary skill, the level of predictability in the art, the amount of direction provided by the inventor, the existence of working examples, and whether the quantity of any necessary experimentation to make or use the invention based on the content of the disclosure is "undue" (*In re Wands*, 858 F.2d 731, 737, 8 USPQ2ds 1400, 1404 (Fed. Cir. 1988)). Furthermore, USPTO does not have laboratory facilities to test if an invention will function as claimed when working examples are not disclosed in the specification. Therefore, enablement issues are raised and discussed based on the state of knowledge pertinent to an art at the time of the invention. And thus, skepticism raised in the enablement rejections are those raised in the art by artisans of expertise.

The Breadth of the Claims and The Nature of the Invention

The claimed invention is directed to an *in vitro* method for producing a high titer antibody producing cell comprising modulating the expression of at least one gene involved in antibody production.

The scope of the method of "suppressing" can be reasonably interpreted to encompass diverse environmental conditions, including temperature and atmospheric variables, and the intracellular and extracellular administration of structurally and materially diverse compositions that have distinctly different modes of operation to directly or indirectly affect the expression,

that is decrease, of alpha-1-antitrypsin (AAT) gene expression and/or function and/or endothelial monocyte activating polypeptide I (EMAP) gene involved in antibody production. When the claims are analyzed in light of the specification, the inventive concept of the instant application is to administer dominant-negative molecules, antisense molecules, ribozymes, knock-out targeting vectors, catalytic antibodies, polypeptide inhibitors, intracellular and/or extracellular antibodies, pharmacologic saturation of substrates or ligands, and molecules of biological or chemical basis that can affect the gene expression profile (page 4, [0012-0013]; page 7, [0022]) to modulate the expression of a gene involved in antibody production. The preferred embodiment of the instantly elected invention, Claim 28, is to suppress the expression of said gene(s) by introducing into the cell a knock-out targeting vector to disrupt the function of said gene(s).

The inventive concept of the instant application is to inhibit or disrupt the expression of alpha-1-antitrypsin (AAT) gene expression and/or function and/or endothelial monocyte activating polypeptide I (EMAP) gene expression and/or function to effect high-titer antibody producing cells (page 7, [0022]). The preferred embodiment of the instantly elected invention, Claim 28, is to introduce into the cell a knock-out targeting vector to disrupt the function of genes encoding alpha-1-antitrypsin (AAT) and/or endothelial monocyte activating polypeptide I (EMAP).

The scope of high titer antibody producing cells encompasses diverse organisms across the prokaryotic and eukaryotic kingdoms, as Applicant has contemplated bacteria, yeast, plants and mammals, for example (page 4, [0010]). The preferred embodiment of the instantly elected invention is a hybridoma cell (Claim 20) and a rodent cell (Claim 72). The claims are broad for encompassing an enormous genus of rodents in the animal kingdom. The art teaches that there are approximately 4,000 rodent species, divided into three major groups or sub-orders, Sciuromorpha, Myomorpha and Hystricomorpha, and more than 30 families. The diversity of instantly claimed rodent genus reasonably encompasses, for example, squirrels, chipmunks, beavers, woodchucks, prairie dogs, hamsters, lemmings, voles, porcupines, capybaras, agoutis, chinchilla, as well as many species whose common names include the term "rat" (columbia.thefreedictionary.com/rodent). It is unclear how the instantly claimed method, specifically using gene knock-out technology, may be performed in cell types that, by definition, do not encode AAT or EMAP genes, e.g. bacteria and yeast, for example.

The Amount of Direction Provided by the Inventor and The Existence of Working Examples

The inventive concept of the instant application is a method to inhibit or disrupt the function of alpha-1-antitrypsin (AAT) and/or endothelial monocyte activating polypeptide I (EMAP) genes to effect high-titer antibody producing cells (page 7, [0022]). The preferred embodiment of the instantly elected invention, Claim 28, is a method to suppress the expression of said gene(s) by introducing into the cell a knock-out targeting vector to disrupt the function of said gene(s). Given the absence in the prior art teaching a mechanism linking AAT and EMAP expression/activity and antibody production, an artisan is dependent upon the instant disclosure to provide specific, not general, guidance.

With regard to the method for enhancing antibody production in a hybridoma cell by introducing a knock-out vector into the cell to inactivate expression of genes encoding AAT and/or EMAP, the specification does not teach an example of the claimed method. Rather, the specification teaches that the H6 hybridoma cell line containing vectors expressing structurally unknown antisense constructs to both the alpha-1-antitrypsin (AAT) gene of SEQ ID NO:1 and the endothelial monocyte-activating polypeptide I (EMAP) gene of SEQ ID NO:2 (page 24, Example 4, Table 2). However, given the absence of a structural disclosure identifying the specific chemical nature of these antisense constructs, the degree of unpredictability in the art regarding antisense RNA methods to decrease the expression of one's gene(s) of interest, the fundamental nature of numerous AAT genes existing in the hybridoma cell, and the novelty of the observed cellular response, one of ordinary skill in the art cannot reasonably predict the phenotype obtained when the individual gene(s) is(are) totally disrupted. Furthermore, method Claim 28 recites "*a* (*emphasis added*) knock-out targeting vector" and the specification does not provide specific active steps to guide an artisan to effectively inactivate all mammalian AAT genes by homologous recombination with one knock-out targeting vector.

The specification discloses that the enhanced antibody production phenotype is obtained when both AAT and EMAP I activities are suppressed. The question then arises whether both activities must be suppressed to yield the desired phenotype or if only one activity is necessary. Applicant discloses having used AAT antiserum that recognizes the peptide QSPIFVGKVVDPTHK (SEQ ID NO:17) to corroborate the antisense experiment (pg 25,

Examples 5-6), and was successful in measuring increased antibody production, thus indicating that suppressing EMAP activity is not necessary *a priori*.

The Examiner notes that the peptide QSPIFVGKVVDPTHK is present (100% identical) in the murine A1AT2 (NP-033270.3) and A1AT4 (NP-033272.1) genes, substantially present (one amino acid difference) in murine A1AT1 (NP-033269.1), A1AT3 (NP-033271.1), A1AT5 (NP-033273.1) and A1AT6 (P81105) genes, and substantially present (three amino acid differences) in the human A1AT gene (see Search Results). Thus, the antiserum experiment does not shed light on the question of which specific AAT protein(s) is responsible for the observed phenotype because up to six murine and one human A1AT polypeptides may have been functionally inactivated by the antiserum.

The specification does not disclose if suppression of EMAP activity alone is sufficient to yield the increased antibody production phenotype. Given that mice encode two distinct genes, NP-058020.1 on chromosome 3 and XP-982480.1 on chromosome 18, the question of functional redundancy raised for AAT is also germane for EMAP I. The specification is silent with evidence demonstrating that inactivation of EMAP alone by antisense or antiserum can also recapitulate the increased antibody production phenotype. The Examiner notes that the antiserum experiment of the instant specification would not address the issue of functional redundancy because both murine polypeptides are recognized by the antiserum directed against the EMAP peptide (pg 26, line 1, SEQ ID NO:18).

The scope of high titer antibody producing cells (claims 19 and 28) encompasses diverse organisms across the prokaryotic and eukaryotic kingdoms, as Applicant has contemplated bacteria, yeast, plants and mammals, for example (page 4, [0010]). It is unclear how the instantly claimed method, specifically using gene knock-out technology, may be performed in cell types that, by definition, do not encode AAT or EMAP genes, e.g. bacteria and yeast, for example. The artisan cannot remove a gene that is not present *a priori* in a given cell and reasonably expect to enhance antibody production in said cell.

With regard to the method for enhancing antibody production in cells associated with hypoimmunoglobulin disease, the specification does not provide an example of introducing a knock-out vector in any cell type associated with hypoimmunoglobulin disease of any organism to inactivate a specific AAT and/or EMAP gene(s) to enhance antibody production.

The State of the Prior Art, The Level of One of Ordinary Skill, and The Level of Predictability in the Art

The art teaches (Forsyth et al, 2003) that alpha-1-antitrypsin (AAT), also known as alpha-1-protease inhibitor, is a member of the rather large (>700 known to date) and functionally diverse family of serine protease inhibitors (SERPINs). Forsyth et al teach that, in striking contrast to the human and bovine genomes wherein AAT is represented by a single gene, four genes are found in the guinea pig and rabbit and individual mouse species possess as many as five AAT genes (page 337, column 2). Furthermore, intraspecific gene number variation is observed, as the *M. domesticus* C57/BL/6J, AJ, and C3H/HeJ laboratory strains express five AAT variants; whereas, the *M. domesticus* AKR/J and DBA/2J laboratory strains express only three AAT variants. Each member of the AAT gene family shares the strongly conserved, protease recognition region that is exposed for interaction with the AAT ligand. Thus, numerous AAT genes exist in the disclosed H6 hybridoma cell line, including other mammalian AAT gene(s), the Applicant-disclosed murine AAT gene of SEQ ID NO:1, and at least two and potentially four, additional ATT murine genes.

With respect to any effect alpha-1-antitrypsin (AAT) activity contributes towards antibody production, the art is largely silent. Jeanin et al teach that exogenously applied AAT potentiates IgE and IgG4 synthesis in human peripheral blood morphonuclear cells (PBMCs) and B-cells, resulting in a 950% increase in IgE production (see Figures 1-3 and Table 1). In contrast to the *trans*-effect taught by Jeanin et al, the art makes no mention of any *cis*-effect AAT expression, or lack thereof, contributes towards antibody production within the given host cell wherein AAT gene expression has been abrogated, down-regulated or disrupted entirely. Rather, the art teaches that the build-up of improperly folded AAT contributes to human disease by impairing protein maturation in the endoplasmic reticulum, polymerizing into protein aggregates and forming intracellular inclusion bodies, and ultimately causing cytotoxicity (Welch et al, 2004). Thus, complete removal of the alpha-1-antitrypsin (AAT) gene(s) in any organism for the production of high-titer antibody producing cells, as disclosed in the specification, is not a routine practice, and despite the general high level of expertise in the art, considerable

unpredictability exists in the field regarding any effect AAT has on antibody production and secretion, especially in light of the organism-dependent diversity of AAT gene number.

Endothelial monocyte-activating polypeptide I (EMAP I) is also known in the art as S100 calcium binding protein A11 (S100A11) and Calgizzarin (Marenholz et al, Biochem. Biophys. Res. Comm. 322: 1111-1122, 2004; pg 1113, Table 1). Santamaria-Kisiel et al (Biochem J. 396:201-214, 2006) teach that the S100 protein family comprises at least 25 members, forming the largest group of EF-hand signaling proteins in humans (Abstract; pg 202, col. 1, S100 proteins). S100 proteins are proposed to have intracellular and extracellular roles in the regulation of many diverse processes such as protein phosphorylation, cell growth and motility, cell-cycle regulation, transcription, differentiation and cell survival (pg 203, col. 1, Biological Roles), wherein S100A10, A11 and A16 have broad tissue distribution (Marenholz et al, pg 1117, col. 2, last ¶).

The murine Calgizzarin gene (a.k.a. S100A11) has been knocked out, and the resulting null mice display no obvious phenotypes (Mannan et al, Molecular Reproduction and Development 66: 431-438, 2003). Mannan et al hypothesize that the lack of a phenotype may be due to functional redundancy or compensation by another member of the S100 gene family (pg 437, col. 1, ¶1), wherein a minor increase in protein level by the hypothesized S100 family member could significantly influence the ability of said family member to function as a mediator in calcium signaling pathways, thereby compensating for the loss of S100A11. Mice encode two distinct genes. NP-058020.1 (S100A11) is located on chromosome 3 and is 100% identical to U41341.1 disclosed in the instant specification. XP-982480.1 is located on chromosome 18. XP-982480.1 was identified in the art quite recently, June 20, 2007, and thus there is no evidence for or against the ability of this essentially identical polypeptide to functionally compensate for S100A11. However, given the striking, nearly identical amino acid sequences between S100A11 and XP-982480.1, differing from S100A11 by one amino acid change; $G_{53} \rightarrow D_{53}$, which lies outside of the Helix I-Helix II and the Helix III-Helix IV S100 and Canonical EF-hands, respectively, it would not be surprising if the two polypeptides are functionally equivalent.

The art teaches that hybridoma technology for the production of antibodies for both research and therapeutic purposes has been in use for almost thirty years, and thus the level of ordinary skill in the art is high (Laffly and Sodoyer, 2005). Laffly and Sodoyer recognize that "the huge demand for large amounts of monoclonal antibodies is currently driving improvement of existing expression systems or the quest for alternative production means" (page 45, column 2, lines 4-7). The art recognizes that the term "hybridoma", as commonly used in the art, represents antibody producing B-cells fused with immortalized myeloma cells, wherein the mammalian species of the B-cells and the mammalian species of the myeloma cells need not be identical, to produce a rapidly and indefinitely growing population of hybridoma cells that will produce antibodies, wherein each specific hybridoma fusion will produce one type of antibody. The specification does not disclose the species derivation of the H6 hybridoma cell; however, Komori et al (1988), for example, teach a human-mouse hybridoma (H6-3C4). The specification does not provide any disclosure regarding the number of existing mammalian AAT genes expressed within the H6 hybridoma cell line.

At the time of filing, the art did not consider the phenotype of a knock-out to be predictable. The art teaches that while the promise of gene targeting had been to reveal the *in vivo* function of a gene of interest, the functional relevance of gene targeting has been questioned because the mutation might lead to an avalanche of compensatory processes (up- or down-regulation of gene products) and resulting secondary phenotypical changes. The art recognizes (Doetschman, 1999) that knock-out targeting vectors require the presence of DNA sequences homologous to the target gene and flanking the disrupted gene cassette to facilitate homologous recombination into the targeted genome, thus replacing the endogenous gene with an exogenous gene fragment and thereby altering the endogenous gene in a pre-specified manner. Doetschmann provides numerous examples of instances in which genes considered well-characterized *in vitro* have produced unexpected phenotypes or indiscernible or no phenotypes in transgenic or knockout mice. Moens et al (1993, Development, 119: 485-499) further teach that different mutations in the same gene can lead to unexpected differences in the phenotype observed. Moens et al. shows that two mutations produced by homologous recombination in two different locations of the *N-myc* gene produce two different phenotypes in mouse embryonic

stem cells, one leaky and one null. A null mutant organism might not only lack the product of a single gene, but might also possess a number of physiological or other processes that have been altered to compensate for the effect of the null mutation (Gerlai, 1996, Trends Neurosci, 19: 177-181, page 177, column 1, paragraph 1). Gerlai teaches an example wherein background genotype can confound the exhibited phenotypes. Targeted disruption of a gene of interest, Gene 1, might lead to changes in expression of alleles b and B for Gene 2. A regulatory change in Gene 2 might lead to different phenotypic changes, depending on which allele (b or B) is present in the organism with the null mutation in Gene 1. The consequences of this problem is that due to this polymorphism in the genetic background, one cannot conclude for certain that a phenotypic change exhibited in a null-mutant resulted from the null mutation or to the genetic background (Gerlai, page 177, column 1, under "Polymorphism in the genetic background might make the results of gene-targeting studies difficult to interpret").

Thus, the art at the time of filing clearly establishes the unpredictability of determining the phenotype of transgenic or knockout conditions even when the activity of the gene has been extensively studied *in vitro*. With respect to the instant invention, Gerlai's teachings indicate that an artisan cannot predict that any gene disruption would necessarily result in a phenotype. And if a phenotype were to result, an artisan cannot predict that the resultant phenotype was the result of the gene disruption. The teachings by Gerlai indicate that phenotypes exhibited by knock-out transgenes can be the result of unrelated factors. The teachings in the art indicate that guidance needs to be given such than an artisan knows how to discriminate what phenotypes are the result of AAT and/or EMAP gene disruption(s) and what are the result of non-specific factors such as genetic background. Given that alpha-1-antitrypsin (AAT) is a member of the rather large genus of serine protease inhibitors (SERPINS) that have a variety of diverse functions in the animal, an artisan cannot predict what biological function occurs in a family of SERPINS such that an artisan would know that the phenotype exhibited in the knockout cell or organism is a result of the gene disruption. The artisan cannot reasonably predict that the phenotype is the result of gene disruption, because the art teaches that non-specific factors, such as genetic background, affect the presence or absence of phenotypes. As such, the specification fails to teach that the phenotypes have a biological relationship with AAT and/or EMAP gene disruption.

With regard to the ability of an artisan to correlate an observed antisense RNA phenotype to a predicted phenotype using targeting vectors that knock-out a targeted gene, Caplen teaches that the RNAi machinery can be saturated, so there will probably be a limit to the number of different genes that can be targeted in a cell at one time (page 1244, column 1). Furthermore, Caplen expresses the importance in recognizing that there is variation in the degree of inhibition mediated by different small interfering RNA sequences which may result in the production of different phenotypes. Thus, the disclosure of a phenotype in response to the expression of a single, structurally undefined antisense molecule (page 24, Example 4, Table 2, discussed below) cannot reasonably predict the phenotype obtained when the individual gene is totally disrupted.

The Quantity of Any Necessary Experimentation to Make or Use the Invention

The substantive technical issue with the instant invention is the realistic possibility of functional redundancy between the related murine and human A1AT family members and the (at least two) murine and human S100A11 family members extant in the murine genome if murine cells (alone or as hybridoma) are to be used in the claimed invention. In the absence of disclosure regarding the structural nature of the antisense oligonucleotides and what specific gene(s) said oligonucleotides inactivate, the artisan would essentially have to knockout by gene targeting five murine A1AT genes, a potential sixth murine A1AT gene (A1AT6), two murine EMAP genes, and in the case of a murine/human hybridoma, an additional human A1AT gene and S100A11 gene. Thus, ten distinct genes must be inactivated by targeted gene disruption. Furthermore, because each genome is diploid, both alleles of each gene must be disrupted, thus requiring twenty independent knockout events because the specification does not teach how to predictably select for cells that are homozygous for each particular AAT and EMAP/S100 gene that has been disrupted. While the art teaches that sequential targeted disruption, e.g. triple knockout, is possible (Robinson et al, PNAS 96: 11452-11457, 1999), the art is silent with respect to generating twenty genomic knockout events in a single cell. The Examiner notes that Robinson et al created triple knock-out animals by starting with embryonic stem cells already heterozygous deficient for two of the desired genes, making the third knockout mutation, creating heterozygous triple knockout animals and obtaining homozygous triple knockout cells via mating. The instant invention is drawn to cells cultured *in vitro*, e.g. hybridoma, and thus a

selection scheme must be set forth to predictably select for homozygosity of each of the ten AAT and EMAP/S100 genes that are to be knocked out.

Therefore, in view of the art recognized diversity in species-specific AAT gene number(s), the silence in the art teaching any role(s) any alpha-1 antitrypsin activity has on antibody production, the breadth of the AAT genes and disruptions in the AAT and/or EMAP/S100 genes claimed, the lack of specific demonstrations in the disclosure teaching method steps of introducing a targeting vector to disrupt the up to ten genes (murine/human hybridoma cell) encoding any AAT, alone or in combination with, EMAP/S100 that will cause increased antibody production, the lack of a null phenotype resulting in high titer antibody production observed by the prior art when AAT and/or EMAP genes are knocked-out in cells or organisms, the unpredictability in determining a knock-out phenotype even when the activity of the gene has been *extensively* (*emphasis added*) studied *in vitro*, the unpredictability in correlating any observed phenotype in a knockout cell or organism with gene disruption as acknowledged by the prior art, and the etiological and pathological diversity of hypoimmunoglobulin disease origins, the quantity of necessary experimentation to make or use the invention as claimed, based upon what is known in the art and what has been disclosed in the specification, will create an undue burden for a person of ordinary skill in the art to demonstrate that the method step of introducing a knock-out targeting vector to inactivate any alpha-1-antitrypsin gene(s), alone or in combination with, endothelial monocyte activating polypeptide I gene(s) in a cell will result in a high-titer antibody producing cell or enhance antibody production in a cell associated with hypoimmunoglobulin disease.

Applicant's Arguments

Applicant argues that:

a) the AAT and EMAP antisense studies described in the instant disclosure provide sufficient basis to predict AAT and EMAP gene knockout phenotypes because an antisense-mediated knockdown phenotype generally correlates with its respective genomic knockout phenotype. While it is generally difficult to predict knockout

phenotype for a given gene, antisense phenotype generally correlates with knockout phenotype (Kline, pg 13, ¶28);

b) there is no expectation of functional redundancy for AAT or EMAP gene products because there is no mention or suggestion in the scientific literature of functional redundancy for AAT or EMAP. Knockout of alpha-1-antitrypsin would most likely not be counteracted by SERPIN family redundancy, including redundancy among the AAT genes, as evidenced by alpha-1-antitrypsin deficiency in humans (Kline, pg 14, ¶31);

c) even if an extant additional AAT gene were present and were to compensate for the loss of the knocked out AAT gene, there is no reason to expect the net effect of enhanced antibody expression to occur;

d) the instant application does not seek to determine the functions of AAT, but instead is directed toward producing a particular phenotype known to be associated with AAT suppression;

e) dual and triple knockdown cells were readily produced using techniques such as sequential targeting, and thus would not require undue experimentation;

f) as of 2003, a vast number of genetic libraries providing the sequences of genes of interest were available to scientists, thus simplifying the step of obtaining the sequence of a gene, as well as upstream and downstream sequences, in a given organism so as to design knock-out vectors;

g) screening for increased antibody production would not require undue experimentation.

Applicant's argument(s) has been fully considered, but is not persuasive.

The determination that "undue experimentation" would have been needed to make and use the claimed invention is not a single, simple factual determination. Rather, it is a conclusion reached by weighing all the...noted factual considerations, specifically the breadth of the claims, the nature of the invention, the state of the prior art, the level of one of ordinary skill, the level of predictability in the art, the amount of direction provided by the inventor, the existence of working examples. *In re Wands,* 858 F.2d at 737, 8 USPQ2d at 1404.

MPEP §2164.06(b) Decision Ruling that the Disclosure was Nonenabling

(A) In *Enzo Biochem, Inc. v. Calgene, Inc.*, 188 F.3d 1362, 52 USPQ2d 1129 (Fed. Cir. 1999), the court held that claims in two patents directed to genetic antisense technology (which aims to control gene expression in a particular organism), were invalid because the breadth of enablement was not commensurate in scope with the claims. Both specifications disclosed applying antisense technology in regulating three genes in *E. coli*. Despite the limited disclosures, the specifications asserted that the "[t]he practices of this invention are generally applicable with respect to any organism containing genetic material which is capable of being expressed ... such as bacteria, yeast, and other cellular organisms." The claims of the patents encompassed application of antisense methodology in a broad range of organisms. Ultimately, the court relied on the fact that (1) the amount of direction presented and the number of working examples provided in the specification were very narrow compared to the wide breadth of the claims at issue, (2) antisense gene technology was highly unpredictable, and (3) the amount of experimentation required to adapt the practice of creating antisense DNA from *E. coli* to other types of cells was quite high, especially in light of the record, which included notable examples of the inventor's own failures to control the expression of other genes in *E. coli* and other types of cells. Thus, the teachings set forth in the specification provided no more than a "plan" or "invitation" for those of skill in the art to experiment using the technology in other types of cells.

With respect to a), the structure of the antisense molecule is undisclosed, and thus there is no evidence that the antisense molecule is specific to a single AAT or EMAP/S100 gene in the hybridoma cell. Thus, one cannot predict the phenotype of a gene knockout because the artisan would not know which of the seven (mouse and human) AAT gene(s), which of the three (mouse and human) EMAP/S100 gene(s), and/or which combination of AAT and EMAP/S100 genes must be inactivated to yield the increased antibody production phenotype. The activity of one specific AAT gene alone as it affects antibody production is not disclosed. Similarly, the activity of one specific EMAP-I/S100 gene alone as it affects antibody production is not disclosed. The disclosed antisense experiment, inactivation of one or more AAT and one or more EMAP/S100 genes, is not commensurate with the scope of the claimed method, specifically: i) cells in which (at least one) AAT gene and (at least one) EMAP/S100 gene are knocked out, ii) cells in which

(at least one) AAT gene is knocked out, or iii) cells in which (at least one) EMAP/S100 gene is knocked out.

With respect to b), no evidence is presented for the lack of functional redundancy, and thus the argument is without support. Applicant appears to mis-comprehend functional redundancy, especially as it applies to AAT. Humans encode one AAT gene, and thus there are no other AAT genes to functionally compensate for that loss. Therefore, the human disease condition caused by the inactivation of the only AAT gene does not speak to the claimed rodent cells comprising three or more A1AT genes. Functional redundancy is usually discovered after the fact and/or per hypothesis-driven experimentation, and at the time of the invention, no genomic knock-out of one or more A1AT genes was described in rodents. Furthermore, the art has recently described the identification of a second S100 gene essentially identical to EMAP-I (one amino acid difference). Clearly, this is a biological reality that cannot be ignored. The scope of the claimed cells is not limited to only those genomes that possess one A1AT gene and one EMAP-I/S100A11 gene so as to obviate functional compensation by closely related gene family members.

With respect to c), the argument is logically inconsistent. The basis of the biological phenomenon of functional redundancy is that the compensating gene effectively replaces the function of the knocked out gene, thereby preventing the manifestation of a phenotype, e.g. enhanced antibody production.

With respect to d), the desired phenotype, specifically enhanced antibody production, is the germane "function" being determined in the instant application. The substantive issue is which AAT gene(s) and what EMAP/S100 gene(s) "function" must be suppressed to yield the desired phenotype?

With respect to e), Applicant's argument is not commensurate in scope with the claimed invention. The artisan would essentially have to knockout by gene targeting five murine A1AT genes (A1T1-5), a potential sixth murine A1AT gene (A1AT6), two murine EMAP genes (NP-058020.1 (S100A11) and XP-982480.1), and in the case of a murine/human hybridoma cell, an additional human A1AT gene and S100A11 gene. Thus, ten distinct genes must be inactivated by targeted gene disruption. Furthermore, because each genome is diploid, both alleles of each gene must be disrupted, thus requiring twenty independent knockout events because the specification

does not teach how to select for cells that are homozygous for each particular AAT and EMAP/S100 gene that has been disrupted. The instant invention is drawn to cells cultured *in vitro*, e.g. hybridoma, and thus a selection scheme must be set forth to predictably select for homozygosity of each of the ten AAT and EMAP/S100 genes that are to be knocked out.

With respect to f), the argument is not commensurate in scope to the claimed invention. The breadth of the claimed cell encompasses diverse organisms across the prokaryotic and eukaryotic kingdoms, as Applicant has contemplated bacteria, yeast, plants and mammals, for example (page 4, [0010]). As discussed above, it is unclear how the instantly claimed method, specifically using gene knock-out technology, may be performed in cell types that, by definition, do not encode AAT or EMAP genes, e.g. bacteria and yeast, for example.

The preferred embodiment of the instantly elected invention is a hybridoma cell (Claim 20) and a rodent cell (Claim 72). The art teaches that there are approximately 4,000 rodent species, divided into three major groups or sub-orders, Sciuromorpha, Myomorpha and Hystricomorpha, and more than 30 families. The diversity of instantly claimed rodent genus reasonably encompasses, for example, squirrels, chipmunks, beavers, woodchucks, prairie dogs, hamsters, lemmings, voles, porcupines, capybaras, agoutis, chinchilla, as well as many species whose common names include the term "rat" (columbia.thefreedictionary.com/rodent). Applicant has provided no evidence that the sequences of AAT and EMAP/S100 genes are known for the rodentia genus. The Examiner reminds Applicant of the recently discovered XP-982480.1 murine gene as an indication of incomplete nucleotide sequence databases pertaining to the long-studied laboratory mouse.

With respect to g), the Examiner finds this argument persuasive.

The substantive issue for the enablement rejection is whether or not the plurality of murine and human AAT and EMAP/S100 genes extant in murine and hybridoma cells, as discussed above, are functionally redundant.

The specification does not disclose whether structurally undisclosed AAT antisense molecule administered to the hybridoma cell inactivates some, all or only a specific mouse AAT gene(s) and the one human gene. Similarly, specification does not disclose whether structurally

undisclosed EMAP-I antisense molecule administered to the hybridoma cell inactivates the two mouse EMAP/S100 genes and the one human S100A11 gene.

If the antisense molecules inactivate more than one AAT gene and more than one EMAP/S100 gene, respectively, then the phenomena of functional redundancy cannot be ignored because the artisan would not know which of the mouse and human AAT gene(s) and/or which of the mouse and human EMAP/S100 genes is(are) responsible for the increased antibody production phenotype.

If the antisense molecules **do not** inactivate more than one AAT gene and more than one EMAP/S100 gene, respectively, then those mouse and/or human AAT genes not inactivated by the structurally undisclosed antisense molecule are, *a priori*, not functionally redundant to the specific AAT or EMAP/S100 gene whose inactivation is responsible for the increased antibody production phenotype. The issue of functional redundancy becomes moot and the remaining issue is reduced to: Which specific mouse and/or human AAT gene(s) and EMAP/S100 gene(s), respectively, must be inactivated to yield the increased antibody production phenotype?

The USPTO does not have the laboratory facilities to ascertain what specific AAT (mouse and human) and EMAP/S100 (mouse and human) genes are inactivated by the structurally undisclosed antisense oligonucleotides. Thus, the burden is shifted to Applicant, who does possess the necessary reagents and facilities to provide evidence of record which AAT gene(s) and which EMAP/S100 gene(s) must be inactivated so as to yield the increased antibody production phenotype.

The simple statements advanced by Applicant that no undue experimentation exists is inadequate as compared to the novelty of the claimed invention, the silence in the art regarding the correspondence between the claimed genes and the disclosed biological activity with respect to antibody production, and the recognized unpredictability in the art regarding gene knock-out technologies, the degree of undue experimentation necessary to knockout up to twenty alleles in a cell so as to recapitulate the disclosed antisense experiment, and the enormous genus of diverse cell types encompassed by the claims.

Conclusion

2. No claim is allowed.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Kevin K. Hill, Ph.D. whose telephone number is 571-272-8036. The Examiner can normally be reached on Monday through Friday, between 9:00am-6:00pm EST.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Joseph T. Woitach can be reached on 571-272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Kevin K. Hill

Joe Woitach
AU 1633